

WHAT IS CLAIMED IS:

1. A method of producing a biologically active recombinant polypeptide in a duckweed plant culture or a duckweed nodule culture, comprising the steps of:
  - 5 (a) culturing within a duckweed culture medium a duckweed plant culture or a duckweed nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is stably transformed to express said biologically active recombinant polypeptide, and wherein said biologically active recombinant polypeptide is expressed from a nucleotide sequence comprising a coding sequence for the polypeptide and an operably linked coding sequence for a signal peptide that directs  
10 secretion of the polypeptide into the culture medium; and
  - (b) collecting said biologically active polypeptide from the duckweed culture medium.
- 15 2. The method of claim 1, wherein said biologically active recombinant polypeptide is secreted into the duckweed culture medium.
3. The method of claim 1, wherein said nucleotide sequence has at least one attribute selected from the group consisting of:
  - 20 (a) duckweed-preferred codons in the coding sequence for said polypeptide;
  - (b) duckweed-preferred codons in the coding sequence for said signal peptide;
  - (c) a translation initiation codon that is flanked by a plant-preferred  
25 translation initiation context nucleotide sequence; and
  - (d) an operably linked nucleotide sequence comprising a plant intron that is inserted upstream of the coding sequence.
4. The method according to claim 3, wherein said duckweed-preferred  
30 codons are *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons..

5        5.        The method according to claim 4, wherein at least one coding sequence selected from the coding sequence for said polypeptide and the coding sequence for said signal peptide comprises between 70-100 % *Lemma gibba*-preferred codons or *Lemma minor*-preferred codons..

10        6.        The method according to claim 3, wherein said plant-preferred translation initiation context nucleotide sequence consists of the nucleotide sequence "ACC" or "ACA", wherein said context is positioned immediately adjacent to of the 5' end of the translation initiation codon.

15        7.        The method according to claim 3, wherein said operably linked nucleotide sequence comprising said plant intron is the sequence set forth in SEQ ID NO:1.

20        8.        A method of producing a biologically active recombinant polypeptide, comprising the steps of:

      (a)        culturing a duckweed plant culture or a duckweed nodule culture, wherein said duckweed plant culture or said duckweed nodule culture is stably transformed to express said biologically active recombinant polypeptide, and wherein said biologically active recombinant polypeptide is encoded by a nucleotide sequence that has been modified for enhanced expression in duckweed, and

      (b)        collecting said biologically active polypeptide from said duckweed plant culture or said duckweed nodule culture.

25        9.        The method of claim 8, wherein said nucleotide sequence that has been modified for enhanced expression in duckweed has at least one attribute selected from the group consisting of:

      (a)        duckweed-preferred codons in the coding sequence for said biologically active recombinant polypeptide;

- (b) a translation initiation codon that is flanked by a plant-preferred translation initiation context nucleotide sequence; and
- (c) an operably linked nucleotide sequence comprising a plant intron that is inserted upstream of the coding sequence.

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10. The method according to claim 9, wherein said duckweed-preferred codons are *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons.

11. The method according to claim 10, wherein the coding sequence  
10 comprises between 70% and 100% *Lemna gibba*-preferred codons or *Lemna minor*-preferred codons.

12. The method according to claim 9, wherein said plant-preferred translation  
15 initiation context nucleotide sequence consists of the nucleotide sequence "ACC" or "ACA", wherein said context is positioned immediately adjacent to the 5' end of the translation initiation codon.

13. The method according to claim 9, wherein said operably linked nucleotide  
sequence comprising said plant intron is the sequence set forth in SEQ ID NO:1.

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14. The method according to claim 1, wherein said duckweed frond culture or duckweed nodule culture expresses and assembles all of the subunits of a biologically active multimeric protein.

25 15. The method according to claim 14, wherein said biologically active multimeric protein is selected from the group consisting of collagen, hemoglobin, P450 oxidase, and a monoclonal antibody.

30 16. The method according to claim 1, wherein the biologically active recombinant polypeptide is a mammalian polypeptide.

17. The method according to claim 16, wherein the mammalian polypeptide is a therapeutic polypeptide.

5 18. The method according to claim 16, wherein the mammalian polypeptide is selected from the group consisting of insulin, growth hormone,  $\alpha$ -interferon,  $\beta$ -interferon,  $\beta$ -glucocerebrosidase,  $\beta$ -glucuronidase, retinoblastoma protein, p53 protein, angiostatin, leptin, monoclonal antibodies, cytokines, receptors, human vaccines, animal vaccines, plant polypeptides, and serum albumin.

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19. The method according to claim 1, wherein the biologically active recombinant polypeptide is  $\alpha$ -2b-interferon.

20. The method according to claim 19, wherein said  $\alpha$ -2b-interferon is human  
15  $\alpha$ -2b-interferon.

21. The method according to claim 20, wherein said human  $\alpha$ -2b-interferon has the amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:5.

20 22. The method according to claim 1, wherein the biologically active recombinant polypeptide is a biologically active variant of  $\alpha$ -2b-interferon, wherein said biologically active variant has at least 80% sequence identity with SEQ ID NO:4 or SEQ ID NO:5.

25 23. The method according to claim 1, wherein the biologically active recombinant polypeptide is an enzyme.

24. The method according to claim 1, wherein said signal peptide sequence is selected from the group consisting of:

30 (a) the human  $\alpha$ -2b-interferon signal peptide;

- 5 (b) the *Arabidopsis thaliana* chitinase signal peptide;  
(c) the rice  $\alpha$ -amylase signal peptide;  
(d) the modified rice  $\alpha$ -amylase peptide;  
(e) a duckweed signal peptide; and  
(f) a signal peptide native to the biologically active recombinant polypeptide.

25. The method according to claim 24, wherein the signal peptide is the rice  $\alpha$ -amylase signal peptide having the sequence set forth in SEQ ID NO:3.

- 10 26. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 1.

- 15 27. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 26, wherein said duckweed plant culture or duckweed nodule culture is selected from the group consisting of the genus *Spirodela*, genus *Wolffia*, genus *Wolffiella*, and genus *Lemna*.

- 20 28. The stably transformed duckweed plant culture or duckweed nodule culture according to claim 27, wherein said duckweed plant culture or duckweed nodule culture is selected from the group consisting of *Lemna minor*, *Lemna miniscula*, *Lemna aequinoctialis*, and *Lemna gibba*.

- 25 29. A nucleic acid molecule comprising a nucleotide sequence encoding an amino acid sequence selected from the group consisting of:  
(a) the amino acid sequence set forth in SEQ ID NO:4;  
(b) the amino acid sequence set forth in SEQ ID NO:5;  
(c) the amino acid sequence of a biologically active variant of the amino acid sequence shown in SEQ ID NO:4, wherein said biologically active

variant has at least about 80% sequence identity with the amino acid sequence set forth in SEQ ID NO:4; and

- 5 (d) the amino acid sequence of a biologically active variant of the amino acid sequence shown in SEQ ID NO:5, wherein said biologically active variant has at least about 80% sequence identity with the amino acid sequence set forth in SEQ ID NO:5;
- wherein said nucleotide sequence comprises duckweed-preferred codons.

30. The nucleic acid molecule of claim 29, wherein said nucleotide sequence  
10 is the nucleotide sequence set forth in SEQ ID NO: 2.

31. A nucleic acid molecule comprising a nucleotide sequence encoding a signal peptide selected from the group consisting of:

- 15 (a) the rice  $\alpha$ -amylase signal peptide amino acid sequence set forth in SEQ ID NO:6; and
- (b) the modified rice-amylase signal peptide amino acid sequence set forth in SEQ ID NO:7;
- wherein said nucleotide sequence comprises duckweed-preferred codons.

- 20 32. The nucleic acid molecule of claim 31, wherein said nucleotide sequence is the nucleotide sequence set forth in SEQ ID NO: 3.

33. A nucleic acid molecule of claim 29, wherein said nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO:5 and further comprises the  
25 signal peptide-encoding nucleotide sequence given in SEQ ID NO:3, and said nucleotide sequence set forth in SEQ ID NO:5 and said signal peptide-encoding nucleotide sequence given in SEQ ID NO:3 are operably linked.

34. The nucleic acid molecule of claim 33, additionally comprising the intron-  
30 comprising nucleotide sequence given in SEQ ID NO:1, wherein said intron-comprising

nucleotide sequence, said signal peptide-encoding nucleotide sequence and said mature human  $\alpha$ -2b-interferon-encoding nucleotide sequence are operably linked.

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